Citation:

Thorrold CA, Letsoalo ME, Dusé AG, Marais E. Efflux pump activity in fluoroquinolone and tetracycline resistant *Salmonella* and *E. coli* implicated in reduced susceptibility to household antimicrobial cleaning agents. *Int J Food Microbiol.* 2007 Feb 15; 113 (3): 315-320. Epub 2006 Nov 27.

PubMed ID: 17126442

Study Design:

Non-randomized trial

Class:

C - Click here for explanation of classification scheme.

Research Design and Implementation Rating:

NEUTRAL: See Research Design and Implementation Criteria Checklist below.

Research Purpose:

To establish if there is a relationship between antibiotic-resistant organisms and reduced susceptibility to the antimicrobial agents found in commonly used household disinfectant products, and whether this could be due to the action of efflux pumps.

Inclusion Criteria:

Researchers selected *Salmonella typhimurium* and *Escherichia coli* samples from poultry, human and other animal sources.

Exclusion Criteria:

Researchers selected samples; therefore, there were no exclusions.

Description of Study Protocol:

Recruitment

- Bacterial strains of *E. coli* (N=9) and *Salmonella* (N=8) were isolated from fresh and frozen chicken products, clinical samples (humans with infectious intestinal disease) and from veterinary institute samples
- Tetracycline- and ofloxacin-resistant samples were derived through further treatment of the isolates with the antibiotics and identified using disk diffusion susceptibility testing on Mueller-Hinton agar plates with reference to National Committee for Clinical Laboratory Standards.

Design

Non-randomized trial.

Statistical Analysis

- Multilevel (hierarchical) linear models were used with strains at level one or lower and groups at level two or higher
- STATA 8.2 was used for result analysis with significance at 5%.

Data Collection Summary:

Timing of Measurements

- Bacteria were grown overnight
- Ethidium bromide accumulation measured continuously to five minutes
- Chemical accumulation measured continuously to seven minutes
- In-use disinfection procedure measured after two minutes in different concentrations of solution.

Dependent Variables

- Efflux pump activity was measured by ethidium bromide accumulation assays
- Chemical accumulation of antimicrobial components in detergents was assayed by the Chapman and Georopapadakou method
- Effectiveness of household detergents on decreasing bacterial counts were recorded.

Independent Variables

- Tetracycline- and ofloxacin-sensitive and resistant *Salmonella* and *E. Coli* strains from various sources
- In-use disinfection procedure was derived from specifically contaminated dishcloths immersed in household disinfectant products (containing sodium salicylate, triclosan, or 2-phenylphenol) at recommended concentration and weaker concentrations of 25% and 50%.

Description of Actual Data Sample:

- *Initial N:* 17 samples (eight *Salmonella,* nine *E. coli*)
- *Attrition (final N):* 17
- *Ethnicity:*
  - Poultry: Six *Salmonella,* one *E. coli*
  - Human: Two *Salmonella,* two *E. coli*
  - Other animals: Two *E. coli*
- *Other relevant demographics:*
  - Tetracycline Resistant: Four *Salmonella,* five *E. coli*
  - Ofloxacin Resistant: Zero *Salmonella,* five *E. coli*
- *Location:* Johannesburg, South Africa.

Summary of Results:

Key Findings

- Active efflux of ethidium bromide was solely associated with antibiotic-resistant organisms, suggesting that efflux mechanisms could be responsible for the antibiotic resistance
- The antibiotic-sensitive bacteria were also more susceptible than the resistant isolates to the household microbial agents at concentrations below that recommended by the manufacturer
- A significant increase in the amount of cell-associated components was seen with antibiotic-resistant *Salmonella* and *E. coli* isolates, indicating that the cells were unable to extrude the agents upon inhibition of the pumps (Z=10.76; P=0.000)
- The higher the concentration of detergent product used, the greater the reduction in bacterial numbers amongst both antibiotic-sensitive and -resistant bacteria
- When detergent product diluted to 25%, more antibiotic-resistant bacteria survived than sensitive isolates (Z=30.20; P=0.000).

Author Conclusion:

While the use of antimicrobial household detergents may assist in reducing bacterial load in the kitchen and elsewhere, incorrect usage could result in selection of bacteria with reduced susceptibility to both antibiotics and antimicrobials. This would be especially undesirable in areas with immuno-compromised individuals.

Reviewer Comments:

- *Strength: Sampling procedures described in detail*
- *Weaknesses: Small sample sizes.*

Research Design and Implementation Criteria Checklist: Primary Research

Relevance Questions		
1.	Would implementing the studied intervention or procedure (if found successful) result in improved outcomes for the patients/clients/population group? (Not Applicable for some epidemiological studies)	Yes
2.	Did the authors study an outcome (dependent variable) or topic that the patients/clients/population group would care about?	Yes
3.	Is the focus of the intervention or procedure (independent variable) or topic of study a common issue of concern to nutrition or dietetics practice?	Yes
4.	Is the intervention or procedure feasible? (NA for some epidemiological studies)	Yes

Validity Questions		
1.	**Was the research question clearly stated?**	Yes
1.1.	Was (were) the specific intervention(s) or procedure(s) [independent variable(s)] identified?	Yes

1.2.	Was (were) the outcome(s) [dependent variable(s)] clearly indicated?	Yes
1.3.	Were the target population and setting specified?	Yes
2.	**Was the selection of study subjects/patients free from bias?**	No
2.1.	Were inclusion/exclusion criteria specified (e.g., risk, point in disease progression, diagnostic or prognosis criteria), and with sufficient detail and without omitting criteria critical to the study?	No
2.2.	Were criteria applied equally to all study groups?	Yes
2.3.	Were health, demographics, and other characteristics of subjects described?	N/A
2.4.	Were the subjects/patients a representative sample of the relevant population?	???
3.	**Were study groups comparable?**	Yes
3.1.	Was the method of assigning subjects/patients to groups described and unbiased? (Method of randomization identified if RCT)	Yes
3.2.	Were distribution of disease status, prognostic factors, and other factors (e.g., demographics) similar across study groups at baseline?	N/A
3.3.	Were concurrent controls used? (Concurrent preferred over historical controls.)	Yes
3.4.	If cohort study or cross-sectional study, were groups comparable on important confounding factors and/or were preexisting differences accounted for by using appropriate adjustments in statistical analysis?	N/A
3.5.	If case control or cross-sectional study, were potential confounding factors comparable for cases and controls? (If case series or trial with subjects serving as own control, this criterion is not applicable. Criterion may not be applicable in some cross-sectional studies.)	N/A
3.6.	If diagnostic test, was there an independent blind comparison with an appropriate reference standard (e.g., "gold standard")?	N/A
4.	**Was method of handling withdrawals described?**	Yes
4.1.	Were follow-up methods described and the same for all groups?	Yes
4.2.	Was the number, characteristics of withdrawals (i.e., dropouts, lost to follow up, attrition rate) and/or response rate (cross-sectional studies) described for each group? (Follow up goal for a strong study is 80%.)	Yes
4.3.	Were all enrolled subjects/patients (in the original sample) accounted for?	Yes
4.4.	Were reasons for withdrawals similar across groups?	N/A

4.5.	If diagnostic test, was decision to perform reference test not dependent on results of test under study?	N/A
5.	**Was blinding used to prevent introduction of bias?**	Yes
5.1.	In intervention study, were subjects, clinicians/practitioners, and investigators blinded to treatment group, as appropriate?	No
5.2.	Were data collectors blinded for outcomes assessment? (If outcome is measured using an objective test, such as a lab value, this criterion is assumed to be met.)	Yes
5.3.	In cohort study or cross-sectional study, were measurements of outcomes and risk factors blinded?	N/A
5.4.	In case control study, was case definition explicit and case ascertainment not influenced by exposure status?	N/A
5.5.	In diagnostic study, were test results blinded to patient history and other test results?	N/A
6.	**Were intervention/therapeutic regimens/exposure factor or procedure and any comparison(s) described in detail? Were interveningfactors described?**	Yes
6.1.	In RCT or other intervention trial, were protocols described for all regimens studied?	Yes
6.2.	In observational study, were interventions, study settings, and clinicians/provider described?	N/A
6.3.	Was the intensity and duration of the intervention or exposure factor sufficient to produce a meaningful effect?	Yes
6.4.	Was the amount of exposure and, if relevant, subject/patient compliance measured?	N/A
6.5.	Were co-interventions (e.g., ancillary treatments, other therapies) described?	N/A
6.6.	Were extra or unplanned treatments described?	N/A
6.7.	Was the information for 6.4, 6.5, and 6.6 assessed the same way for all groups?	N/A
6.8.	In diagnostic study, were details of test administration and replication sufficient?	N/A
7.	**Were outcomes clearly defined and the measurements valid and reliable?**	Yes
7.1.	Were primary and secondary endpoints described and relevant to the question?	Yes
7.2.	Were nutrition measures appropriate to question and outcomes of concern?	Yes
7.3.	Was the period of follow-up long enough for important outcome(s) to occur?	Yes
7.4.	Were the observations and measurements based on standard, valid, and reliable data collection instruments/tests/procedures?	Yes

7.5.	Was the measurement of effect at an appropriate level of precision?	Yes
7.6.	Were other factors accounted for (measured) that could affect outcomes?	No
7.7.	Were the measurements conducted consistently across groups?	Yes
8.	**Was the statistical analysis appropriate for the study design and type of outcome indicators?**	Yes
8.1.	Were statistical analyses adequately described and the results reported appropriately?	Yes
8.2.	Were correct statistical tests used and assumptions of test not violated?	Yes
8.3.	Were statistics reported with levels of significance and/or confidence intervals?	Yes
8.4.	Was "intent to treat" analysis of outcomes done (and as appropriate, was there an analysis of outcomes for those maximally exposed or a dose-response analysis)?	N/A
8.5.	Were adequate adjustments made for effects of confounding factors that might have affected the outcomes (e.g., multivariate analyses)?	No
8.6.	Was clinical significance as well as statistical significance reported?	Yes
8.7.	If negative findings, was a power calculation reported to address type 2 error?	No
9.	**Are conclusions supported by results with biases and limitations taken into consideration?**	No
9.1.	Is there a discussion of findings?	Yes
9.2.	Are biases and study limitations identified and discussed?	No
10.	**Is bias due to study's funding or sponsorship unlikely?**	Yes
10.1.	Were sources of funding and investigators' affiliations described?	Yes
10.2.	Was the study free from apparent conflict of interest?	Yes